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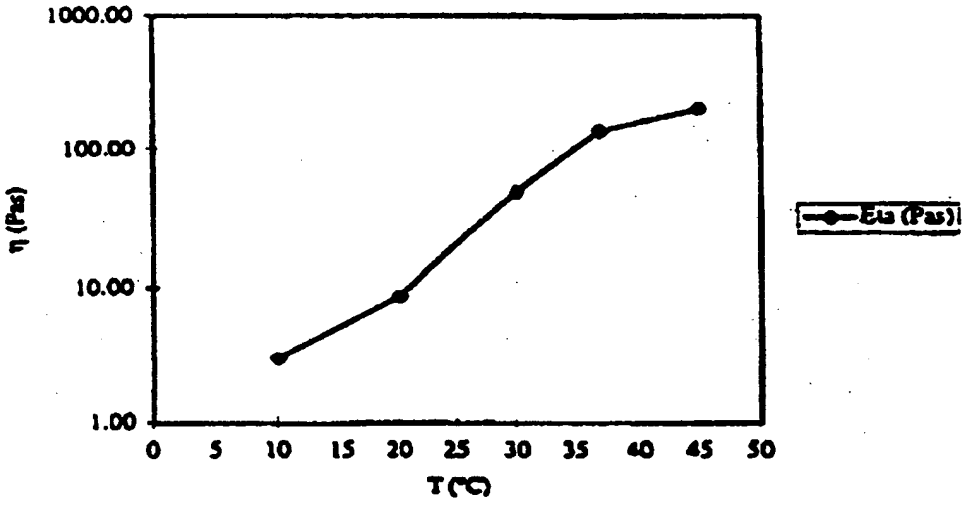

(57) Abstract

Viscoelastic compositions are described comprised of esters of hyaluronic acid which are uniquely advantageous because their viscoelastic characteristics may vary according to shearing stress and temperature.

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ESTER DERIVATIVES OF HYALURONIC ACID WITH VISCOELASTIC PROPERTIES AND THEIR USE IN THE BIOMEDICAL AND HEALTHCARE FIELD

Background And Field Of The Invention

The present invention concerns particular ester derivatives of hyaluronic acid which can vary in their viscosity according to the shearing stress and the temperature. These derivatives, which may be in association with drugs and/or biologically active substances, may be used to advantage for the preparation of biomedical and healthcare compositions which, according to the pattern followed by their rheological properties as the above parameters vary, prove to be particularly suitable in given surgical fields or for the transport and release of drugs.

There are known viscoelastic preparations in the form of gels (EP0466300) constituted by natural and synthetic polymers including hyaluronic acid, the salts thereof, derivatives of hyaluronic acid crosslinked by means of suitable agents such as vinyl sulphone, other glycosaminoglycans and their use in viscoelastic surgery. The compositions containing these derivatives can vary their viscosity according to the shearing stress applied.

The present inventors have surprisingly found that the viscosity characteristics of ester derivatives of hyaluronic acid vary, not only according to variations in shearing stress, but also according to temperature changes. This characteristic can now by virtue of the present invention, be utilized to design particular products based upon these variations in viscosity characteristics.

Brief Description Of The Drawings

Figures 1-4 graph the viscosity characteristics of four different hyaluronic acid esters at varying temperatures.

Figures 5-8 graph the results of tests to measure the pattern of storage modulus (G') and loss modulus (G") for the HA esters under oscillatory strain.

Figures 9-12 show the viscosity pattern of HA esters as the temperature varies.

Figures 13-14 show viscosity values of two HA esters at various temperatures.

Detailed Description Of The Invention

The ester derivatives of hyaluronic acid which are used to advantage in the present invention are particularly those described in EP0216453. These derivatives (also called "HA esters"), besides varying their viscosity according to variations in the shearing stress being applied, show, surprisingly, an increasing or decreasing viscosity pattern as the temperature rises.

The ester derivatives used in the present invention as described in EP0216453, are particularly esters of hyaluronic acid with aliphatic, araliphatic, cycloaliphatic or heterocyclic alcohols, in which all or any of portion of the carboxylic acid groups of the hyaluronic acid are esterified, with the remaining carboxy groups optionally being salified.

Alcohols of the aliphatic series to be used as esterifying components of the carboxylic groups of hyaluronic acid according to the present invention are for example those with a maximum of 34 carbon atoms, which may be saturated or unsaturated and which may possibly also be substituted by other free functional or functionally modified groups, such as amine, hydroxyl, aldehyde, ketone, mercaptan or carboxyl groups or by groups derived from these, such as hydrocarbyl or dihydrocarbylamine groups (from now on the term "hydrocarbyl" will be used to refer not only to monovalent radicals of hydrocarbons such as the C_nH_{2n+1}) type, but also bivalent or trivalent radicals, such as "alkylenes" C_nH_{2n} or "alkylidense" C_nH_{2n}), ether or ester groups, acetal or ketal groups, thioether or thioester groups, and esterified carboxyl or carbamide groups and carbamide substituted by one or more hydrocarbyl groups, nitrile groups or by halogens.

Of the above mentioned groups containing hydrocarbyl radicals, these are preferably lower aliphatic radicals, such a alkyls, with a maximum of 6 carbon atoms. Such alcohols may also be interrupted in the carbon atom chain by heteroatoms, such as oxygen, nitrogen and sulfur atoms. Preferred are alcohols substituted with one or two of the said functional groups.

Alcohols of the above mentioned groups which are preferably to be used within the bounds of the present invention are those with a maximum of 12, and especially 6 carbon atoms, and in which the hydrocarbyl atoms in the above

mentioned amine, ether, ester, thioether, thioester, acetal, ketal groups represent alkyl groups with a maximum of 4 carbon atoms, and also in the esterified carboxyl or substituted carbamide groups the hydrocarbyl groups are alkyls with the same number of carbon atoms and in which in the amine or carbamide groups may be alkylenamine or alkylencarbamide groups with a maximum of 8 carbon atoms. Of these alcohols special mention should be given to those which are saturated and not substituted such as the methyl, ethyl, propyl, and isopropyl alcohols, normal butyl alcohol, isobutyl alcohol, tertiary butyl alcohol, the amyl, pentyl, hexyl, octyl, nonyl and dodecyl alcohol and, above all, those with a linear chain, such as normal octyl and dodecyl alcohols. Of the substituted alcohols of this group, the bivalent alcohols should be listed, such as ethyleneglycol, propyleneglycol and butyleneglycol, the trivalent alcohols such a glycerin, the aldehyde alcohols such as tartronic alcohol, the carboxylic alcohols such as lactic acids, for example glycolic acid, malic acid, the tartaric acids, citric acid, the aminoalcohols, such as a normal aminoethanol, aminopropanol, normal aminobutanol and their dimethylated and diethylated derivatives in the amine function, choline, pyrrolidinylethanol , piperidinylethanol, peperazineylahanol and the corresponding derivatives of normal propylorl or normal butyl alcohol, monothioethyleneglycol or its alkyl derivatives, such as the ethyl derivative in the mercaptan function.

Of the higher saturated aliphatic alcohols the following should be mentioned: cetyl alcohol and myricyl alcohol, the higher unsaturated alcohols with one or two double bonds, are especially important, such as especially those contained in many essential oils and with affinity to terpene, such as citronellol, geraniol, nerol, nerolidol, linalool, farnesol, phytol. Of the unsaturated lower alcohols, useful are allyl alcohol and propargyl alcohol. Of the araliphatic alcohols special attention should be given to those with only one benzene residue and in which the aliphatic chain has a maximum of 4 carbon atoms, which the benzene residue can be substituted by between 1 and 3 methyl or hydroxyl groups or by halogen atoms, especially by chlorine, bromine and iodine, and in which the aliphatic chain may be substituted by one or more functions chosen from the group containing fee amine groups or mono - or dimethylated or by pyrrolidine or piperidine groups. Of these alcohols especially preferred are benzyl alcohol and phenetyl alcohol.

The alcohols of the cycloaliphatic or aliphaticcycloaliphatic series may derive from mono- or polycyclic hydrocarbons, may preferably have a maximum of 34 carbon atoms, may be unsubstituted and may contain one or more substituents, such as those mentioned above for the aliphatic alcohols. Of the alcohols derived from cyclic monoannular hydrocarbons, useful are those with a maximum of 12 carbon atoms, the rings with preferably between 5 and 7 carbon atoms, which may be substituted for example by between one and three lower alkyl groups, such as methyl, ethyl, propyl or isopropyl groups. As specific alcohols of this group the following can be mentioned: cyclohexanol, cyclohexanediol, 1,2,3 cyclohexanetroil and 1,3,5 cyclohexanetriol (phloroglucitol), inositol, and the alcohols which derive from p-methane such as carvomenthol, menthol, and α-γ terpineol, 1-terpineol, 4-terpineol and piperitol, or the mixture of these alcohols known as "terpineol", 1,4- and 1,8 terpin. Of the alcohols which derive from hydrocarbons with condensed rings, such as those of the thujane, pinane or comphane, the following can be mentioned: thujanol, sabinol, pinol hydrate, D and L-borneol and D and L-isoborneol.

In the partial esters, the non-esterified carboxylic groups may be kept free or may be salified. For the formation of such salts the bases are chosen according to the cirterion of these for which the product is intended. It is possible to form inorganic salts deriving from alkaline metals, such as potassium and especially sodium and ammonium, or deriving from alkaline earth metals, such as calcium, or magnesium or aluminum salts.

Particularly interesting are the salts with organic bases, especially nitrogenized bases and therefore aliphatic, arylaliphatic. cycloaliphatic or heterocyclic amines.

As noted above the viscosity variation characteristics of the hyaluronic acid esters can be utilized to design products requiring such variation. For example, the propyl ester of hyaluronic acid with 50% of its carboxy groups esterified presents a pattern of increasing viscosity as the temperature rises (Figure 1), while butyl, benzyl, and octyl esters with 50% of their carboxy groups esterified present a decreasing pattern (Figures 2, 3 and 4). This result, therefore, provides biocompatible compositions able to vary their rheological properties when they are

transferred from outside to inside the organism, because of the change of temperature.

According to their intended uses, compositions of said esters can be prepared, possibly also in association with autocrosslinked esters of hyaluronic acid, as described in EP 0341745, with drugs or biologically active substances, monitoring the viscosity values outside and inside the human body, varying the following parameters:

- the type of alcohol bound to the hyaluronic acid carboxyls;
- the percentage of esterification;
- the type of salt used for salification of the non-esterified carboxy groups;
- the concentration of the components.

By modulating these parameters, it is possible to obtain compositions which are easily injected into the organism and which, once inside the organism, undergo an increase in viscosity, reaching values at which they can perform their function. This behavior can be exploited to advantage in the prevention of post-surgical adhesions, in viscosupplementation and in filling artificial organs.

The derivatives, on the other hand, which present high viscosity compared to that of the human body, can be used to advantage in the preparation of viscoelastic compositions, such as those for ophthalmic use, which release gradually, in situ, the active principle contained therein, as their viscosity decreases on account of the increase in temperature.

As noted, the HA esters can also be utilized in combination with so-called "autocrosslinked" esters of hyaluronic acid, namely those described in EP 0341745.

These derivatives are inter and/or intramolecular esters of hyaluronic acid, in which a part or all of such functions are esterified with hydroxyl groups of the same molecule and/or of different molecules of the acid polysaccharide, thus forming lactone or intermolecular ester bonds. These "inner" esters in which there is no intervention by OH groups of other alcohols can also be defined as "auto-crosslinked polysaccharides", since the formation of a mono- or polymolecular cross-link is the consequence of the above-mentioned internal esterification. The adjective "cross-linked" refers to the crosswise connections between the carboxyls and hydroxyls of

the polysaccharide molecules. The inner esters can be total or partial, depending on whether all or only part of the carboxy functions are esterified in the above manner. In the partial inner esters, further carboxy functions can be either totally or partially esterified with monovalent or polyvalent alcohols, thus forming "external" ester groups, and in the partial esters of both these ester groups the non-esterified carboxy functions may be free or salified with metals or organic bases.

Esterification between different hyaluronic acid molecules consequently increases their molecular weight, which can be roughly doubled or multiplied according to the number of molecules involved in the crosslinking. The degree of "polymerization" varies according to the conditions used in the preparation procedure described hereafter, such as temperature and, reaction duration. Even though it is impossible to ascertain the ratio between the two types of ester bonds, an approximate representation can be made on the basis of the molecular weight, this being proportional to the to the number of molecules of the polysaccharide aggregate of the above-said bonds of intermolecular inner esters.

The autocross-linked products may possess all the carboxy functions in the form of an inner ester, or only on aliquot part of the same. In these particle inner esters the percentage of "cross-links" varies preferably between 1 and 60%, and especially between 5 and 30% of the number of carboxy groups in the acidic polysaccharides.

Of the derivatives of the present invention, particularly interesting are the ones able to undergo a variation in their rheological properties according to the temperature over relatively short periods of time (less than two hours) to be used in the surgical fields in which transition times of said parameters are important, such as in ophthalmology. Moreover, said derivatives, once inserted into the organism, continue to have viscoelastic properties which depend upon the shearing stress being applied.

The viscoelastic esters of hyaluronic acid according to the present invention can be used to advantage in ophthalmology, for example in viscosupplementation of the vitreous, in arthroscopic surgery, for example as lubricants or in viscosupplementation of the joints, in maxillofacial surgery, for example as materials for injection to fill wrinkles, in the substitution of soft tissues and for the growth of

tissues, in neurosurgery and in general surgery for the reconstruction of organs and organ parts and in the prevention of post-surgical adhesions, as materials for filling artificial prostheses, for example as substitutes for silicone gel in breast implants and artificial testicles, in urology, for example for the preparation of urological catheters and in oncology.

Moreover, said compounds can be used to advantage for the preparation of pharmaceutical forms involving the controlled release of drugs and/or of biologically active substances such as peptides, enzymes, proteins or fragments thereof and polynucleotides for use in the sector of vaccination. The derivatives according to the present invention can also be used to transport and release biologically active substances used in the treatment of disorders associated with genetic defects such as those which depend on enzymatic hypo- or hyper-activity due to defects of the gene that encodes for a given enzyme, deforming diseases of genetic origin and hereditary diseases.

According to the invention the hyaluronic acid esters, can therefore be utilized in combination with a variety of biologically or pharmacologically active substances, including those active substances useful in the fields of ophthalmology, gynecology, argiology and neurology. Such active substances can be anti-infective agents, antibiotics, antimicrobials, anti-inflammatory agents, cytostatic, cytotoxic. antiviral and anesthetic agents and growth factors. The esters can also be combined with cells such as chondrocytes, osteocystes, fibroblasts, keratinocytes, adipocytes, muscle cells, nerve cells, from the centrals peripheral nervous system, endothelial cells, hematopoietic cells, glandular cells and stem cells. The viscoelastic HA ester derivatives in the form of microspheres or nanospheres, in association with radioactive substances and nonradioactive substances, can be used in contrast systems, as labels for in vivo diagnostics, to identify and cure tumoral or damaged tissues.

Thus, particular ester derivatives of hyaluronic acid, possibly in association with other polymers, such as the autocrosslinked derivatives of hyaluronic acid and/or drugs and/or biologically active substances can be used for the preparation of biomedical and healthcare compositions with the ability to vary their viscosity as the shearing stress being applied and the temperature change. Said compositions,

according to the ester derivative of hyaluronic acid which is used, undergo an increase or decrease in their viscosity when they are introduced into the organism because of the increase in temperature and moreover they maintain their viscoelasticity with the shearing stress and temperature of the body. The compositions according to the present invention can be used to advantage in surgery, for the transport and release of drugs and biologically active substances.

BIOLOGICAL EVALUATIONS

The rheological properties of the ester derivatives of hyaluronic acid were assessed using HAAKE RV 20 and RS 100 rheometers which function at shearing stress, frequency sweep and temperature gradients.

Example 1

Preparation of the tetrabutylammonium salt of hyaluronic acid (molecular weight 200 KDa) from sodium hyaluronate

69 g of sodium hyaluronate are solubilized in 4 l of H_2O and then passed through a column previously filled with Dowex M15 Resin in "TBA-Form".

After freeze-drying, 100 g of tetrabutylammonium salt of hyaluronic acid (200 KDa) is obtained, which is then packed in food-grade plastic bags.

Example 2

Preparation of HYAFF9 p50 (50% ester of hyaluronic acid with 1-propylbromide) from the tetrabutylammonium salt of hyaluronic acid (200 KDa).

16 g of tetrabutylammonium salt of hyaluronic acid (200 KDa) are solubilized in 800 ml of NMP (N-methylpyrrolidone) so as to obtain a concentration of 20 mg/ml.

The quantity of alkylating agent necessary (1.2 ml), calculated on the basis of the desired degree of esterification (50%), is diluted with 10 ml of NMP and added slowly to the solution of hyaluronic acid tetrabutylammonium salt. The solution is left for at least 60 hours at 37°C.

Lastly, the solution is unloaded from the reactor and 6 g of NaCl is added in a saturated solution. This is then agitated for half an hour, precipitated with 5 volumes of acetone, and the precipitate is filtered and washed with acetone/H_2O at a ratio of 80:20 until testing with A_gNO_3 gives a negative response.

Drying at 30°C for at least 48 hours yields 10.1 g of product.

Example 3

Preparation of HYAFF10 p50 (50% ester of hyaluronic acid with 1-butylbromide) from the tetrabutylammonium salt of hyaluronic acid (200 KDa)

15 g of tetrabutylammonium salt of hyaluronic acid are solubilized in 750 ml of NMP(N-methylpyrrolidone) so as to obtain a concentration of 20 mg/ml.

The quantity of alkylating agent necessary (1.35 ml), calculated on the basis of the desired degree of esterification (50%), is diluted with 10 ml of NMP and added slowly to the solution of hyaluronic acid tetrabutylammonium salt. The solution is left to react for at least 60 hours at 37°C.

The solution is unloaded from the reactor and 6 g of NaCl is added in a saturated solution. This is then agitated for half an hour, precipitated with 5 volumes of acetone, and the precipitate is filtered and washed with acetone/H_2O at a ratio of 80:20 until testing with A_gNO_3 gives a negative response.

Drying at 30°C for at least 48 hours yields 9.1 g of product.

Example 4

Preparation of HYAFF11 p50 (50% ester of hyaluronic acid with benzylbromide) from the tetrabutylammonium salt of hyaluronic acid (200 KDa)

15 g of tetrabutylammonium salt of hyaluronic acid are solubilized in 750 ml of NMP (N-methylpyrrolidone) so as to obtain a concentration of 20 mg/ml.

The quantity of alkylating agent necessary (1.47 ml), calculated on the basis of the desired degree of esterification (50%), is diluted with 10 ml of NMP and added slowly to the solution of hyaluronic acid tetrabutylammonium salt. The solution is left to react for at least 60 hours at 37°C.

The solution is unloaded from the reactor and 6 g of NaCl is added in a saturated solution. This is then agitated for half an hour, precipitated with 5 volumes of acetone, and the precipitate is filtered and washed with acetone/H_2O at a ratio of 80:20 until testing with A_gNO_3 gives a negative response.

Drying at 30°C for at least 48 hours yields 9.1 g of product.

Example 5

Preparation of HYAFF17 p50 (50% ester of hyaluronic acid with 1-octylbromide) from the tetrabutylammonium salt of hyaluronic acid (200 KDa)

10 g of tetrabutylammonium salt of hyaluronic acid are solubilized in 500 ml of NMP (N-methylpyrrolidone) so as to obtain a concentration of 20 mg/ml.

The quantity of alkylating agent necessary (1.42 ml), calculated on the basis of the desired degree of esterification (50%), is diluted with 10 ml of NMP and added slowly to the solution of hyaluronic acid tetrabutylammonium salt. The solution is left to react for at least 60 hours at 37°C.

The solution is unloaded from the reactor and 4 g of NaCl is added in a saturated solution. This is then agitated for half an hour, precipitated with 5 volumes of acetone, and the precipitate is filtered and washed with acetone/H_2O at a ratio of 80:20 until testing with A_gNO_3 gives a negative response.

Drying at 30°C for at least 48 hours yields 4.7 g of product.

Example 6

Preparation of concentrated aqueous solutions of the ester derivatives of hyaluronic acid

The ester derivatives of hyaluronic acid were diluted at different concentrations, according to the substituent, with water obtained by reverse osmosis so as to obtain viscous solutions.

20 ml of solution per derivative was prepared, at the following concentrations, for example:

Ester	Concentration (mg/ml)
HYAFF9 p50	150
HYAFF10 p50	120
HYAFF11 p50	50
HYAFF17 p50	20

Solubilization is performed by pouring the ester derivatives into water slowly while gently agitating, to avoid the formation of lumps of solute. The products are stored at +4°C until ready to be used.

RHEOLOGICAL MEASUREMENTS

Viscosity measurements were made for each product at 10, 20, 30. 37 and 45°C. The results for Hyaff9 p50, Hyaff10 p50, Hyaff11 p50 and Hyaff17 p50 are shown in the following Tables 1-4, respectively and the flow curves are shown in the corresponding Figures 1-4.

Table 1 - Hyaff9 p50

T(°C)	η(Pa s)
10	3.02
20	8.42
30	49.34
37	137.30
45	202.90

Table 2 - Hyaff10 p50

T(°C)	η(Pa s)
10	393.10
20	479.73
30	309.97
37	79.97
45	17.42

Table 3 - Hyaff11 p50

T(°C)	η(Pa s)
10	295.20
20	280.43
30	224.20
37	143.27
45	26.27

Table 4 - Hyaff17 p50

T(°C)	η(Pa s)
10	295.20
20	280.43
30	224.20
37	143.27
45	26.27

Oscillatory measurements were then also made for each product, and the results are shown in Figures 5-14. Figures 5-8 represent the pattern of the storage modulus (G') and loss modulus (G"), of the HA esters when the material undergoes oscillatory applied strain, increasing the oscillation frequency over time. Theoretically, a completely viscous material should present a storage modulus of G' = 0, a high loss modulus (G") and a G' and G" which is independent of the oscillation frequency. Conversely, a completely elastic material should present a loss modulus of G" = 0, a high storage modulus (G') and a G' and G" patter which is dependent upon the oscillation frequency.

The HYAFFs analyzed herein, being viscoelastic, that is, having both of the two above characteristics, present intermediate G' and G" values.

FIG 5: G" > G' and the modulus patters are dependent upon the frequency of oscillation (progressive pattern). The viscous characteristic of HYAFF9 p50, 150 mg/ml, is therefore more marked than its elastic characteristic.

FIG 6: G" ≡ G' and the modulus patterns therefore do depend upon the oscillation frequency. It can therefore be said that HYAFFF 10, 120 mg/ml, is more viscoelastic than the HYAFF9 p50, 150 mg/ml, in the previous figure.

FIG 7: G" < G' and the modulus patterns depend only slightly on the oscillation frequency. HYAFF 11, 50 mg/ml has more marked elastic characteristics than the previous materials.

FIG 8: G" > G' and the modulus patterns are dependent upon the oscillation frequency. The viscous characteristic of HYAFF 17 p 50, 25 mg/ml, is more marked than its elastic characteristic.

Figures 9-12 show the viscosity pattern of the material as the temperature varies and when oscillatory applied strain is applied at constant frequency. The results in Figures 9-12 confirm those in Figures 1-4 which refer to continuous viscosity measurements as the temperature is varied, applying a shear rate of D = 1sec.$^{-1}$. Figures 13-14 show the viscosity curves at various temperatures (10, 20, 30, 37, 45°C) as the shear rate is varied (D). The graphs shown in Figures 1 and 3 were based on the viscosity values at the various temperatures as shown in Figures 13 and 14 respectively, at a shear rate of D = 1sec.$^{-1}$ (viscosity patterns of HYAFF 9 p50, 150 mg/ml and HYAFF 11 p50, 50 mg/ml at varying temperatures, applying a shear rate of D = 1sec.$^{-1}$).

The invention being thus described, it is clear that these compositions can be modified in various ways. Such modifications are not to be considered as divergences from the spirit and purposes of the invention and any modification which would appear evident to an expert in the field comes within the scope of the following claims:

CLAIMS

1) A viscoelastic composition comprising an ester derivative of hyaluronic acid with an alcohol. optionally in association with another polymer and/or drug and/or biologically active or functional substance, said ester derivative being characterized by an ability to modify its rheological properties according to variations in the temperature and shearing stress being applied.

2) The viscoelastic composition according to claim 1, wherein the viscosity increases or decreases as the temperature rises following administration into an organism.

3) The viscoelastic composition according to claim 1 or 2, wherein said ester is able to maintain its viscoelastic properties at the shearing stress at the application site.

4) The viscoelastic composition according to any one of claims 1-3, having the ability to modify its rheological properties as a result of variations in the temperature, in under two hours.

5) The viscoelastic composition according to any one of claims 1-4, wherein said other polymers are autocrosslinked derivatives of hyaluronic acid.

6) The viscoelastic composition according to any one of claims 1-5, wherein the biologically active substance is selected from peptides, enzymes, proteins or their fragments, polynucleotides and growth factors.

7) The viscoelastic composition according to any one of claims 1-6, wherein the ester derivative of hyaluronic acid is the propyl, butyl, octyl or benzyl ester of hyaluronic acid with 50% esterification.

8) The viscoelastic composition according to any one of claims 1-5, wherein the biologically functional material is selected from cells, chondrocytes, osteocytes, fibroblasts, keratinocytes, adipocytes, muscle cells, nerve cells from the

central and peripheral nervous systems, endothelial cells, haematopoietic cells, glandular cells and stem cells.

9) Use of the viscoelastic composition according to any one of the previous claims, in the form of microspheres or nanospheres for use as labels, possibly in association with radioactive or nonradioactive substances, in contrast systems, in in vivo diagnostics, for the identification and cure of tumoral tissues or tissues with lesions.

10) Use of a viscoelastic composition of an ester derivative of hyaluronic acid for the preparation of a viscoelastic composition for biomedical or healthcare uses.

11) Use of the viscoelastic compositions according to any one of claims 1-6 in surgery.

12) Use of the viscoelastic compositions according to any one of claims 1-6, for the transport and release of drugs and biologically active substances.

13) Use of a viscoelastic composition according to any one of claims 1-6, in ophthalmology, in viscosupplementation of the vitreous, in arthroscopic surgery, in maxillofacial surgery, in aesthetic surgery, in the substitution of soft tissues or the growth of tissues, in neurosurgery, in the reconstruction of organs or their parts, in the prevention of post-surgical adhesions, as a filler for artificial prostheses, in urology and in oncology.

FIGURE 1

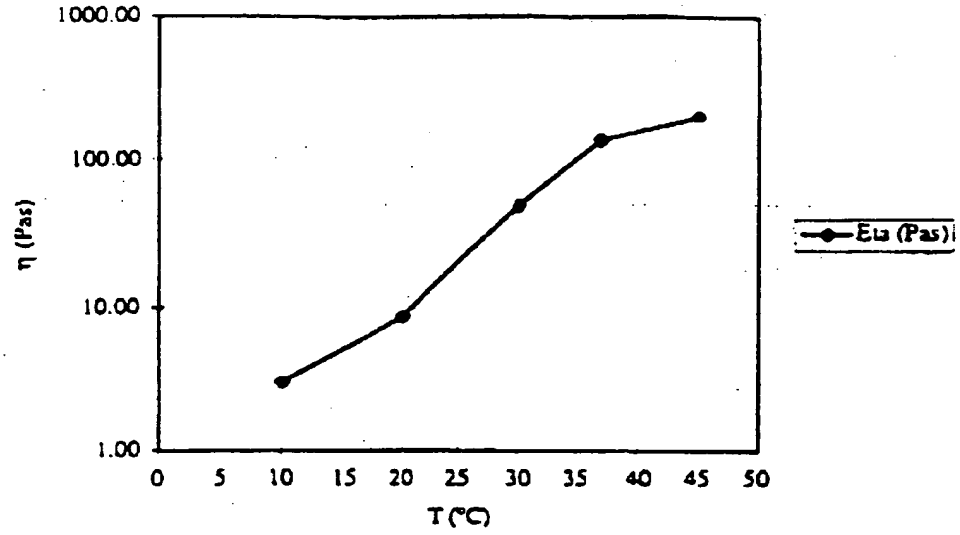


FIGURE 2

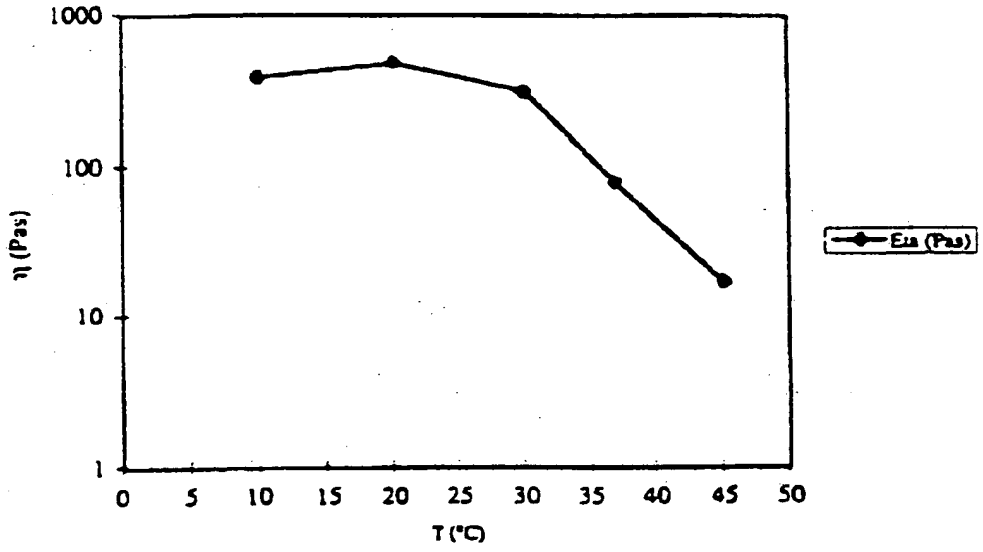


FIGURE 3

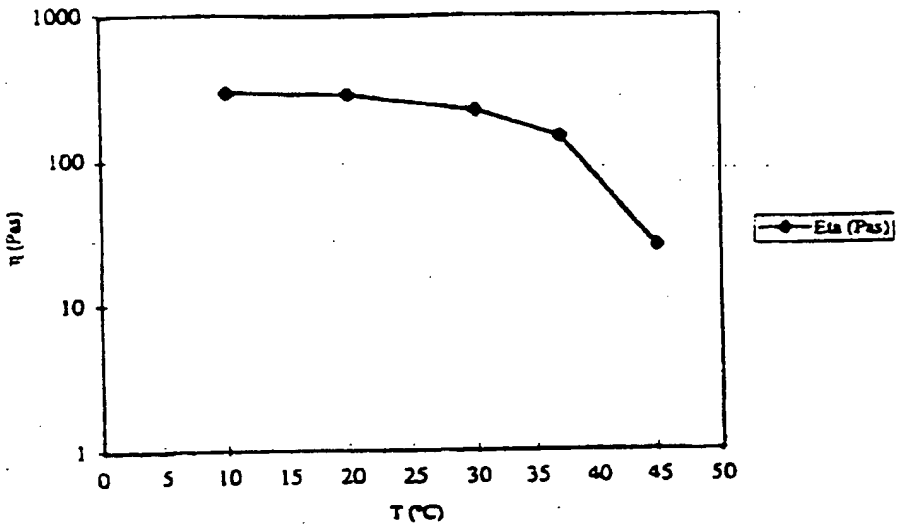
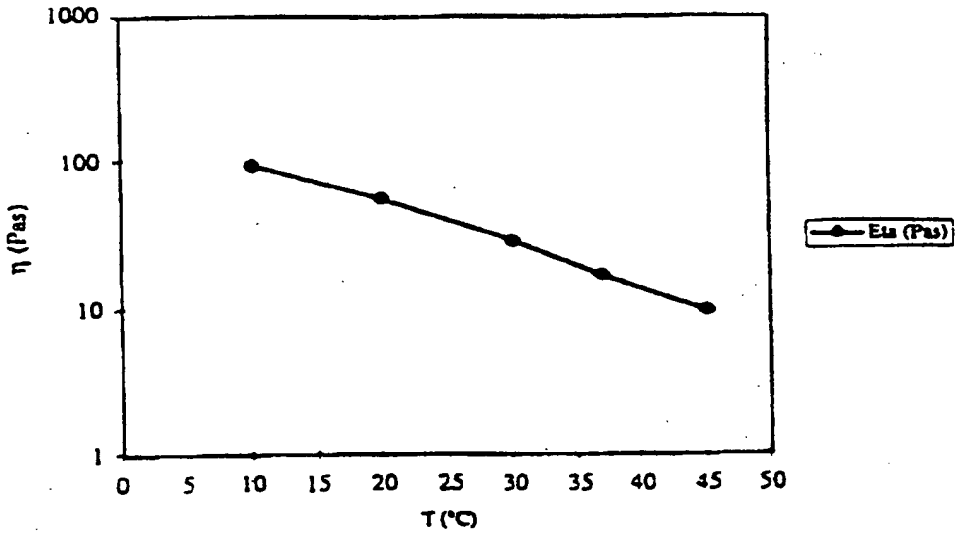


FIGURE 4



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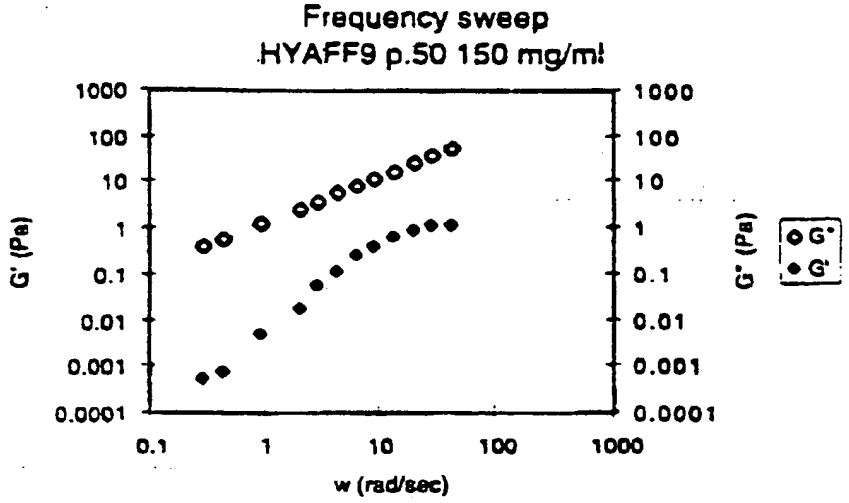


Figure 6

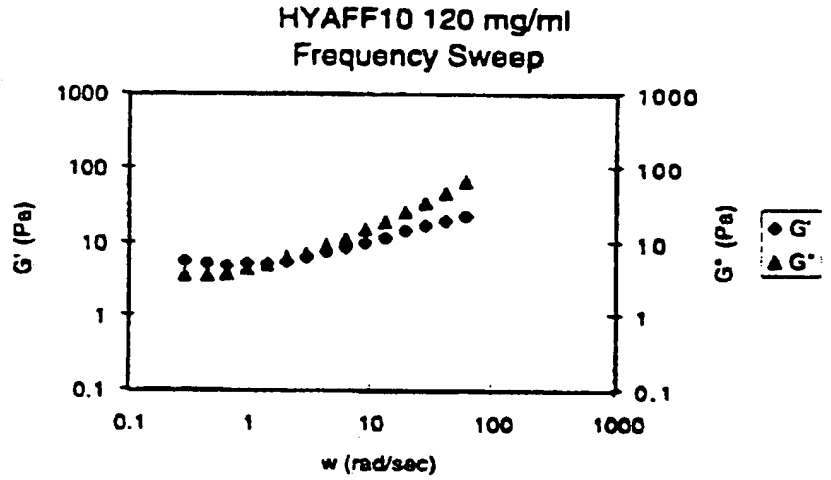


Figure 7

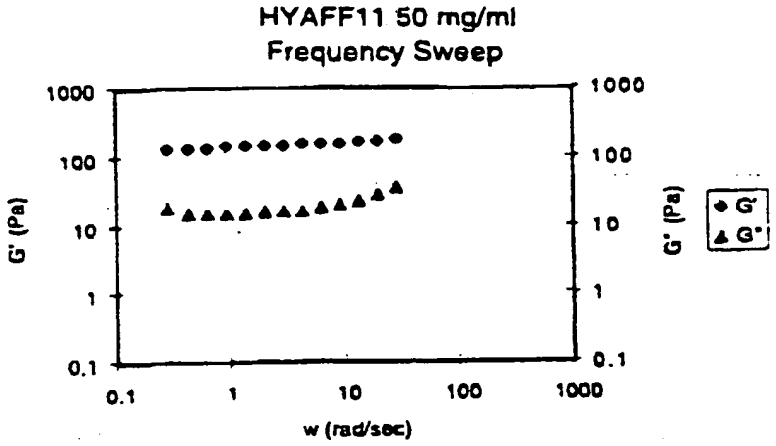


Figure 8

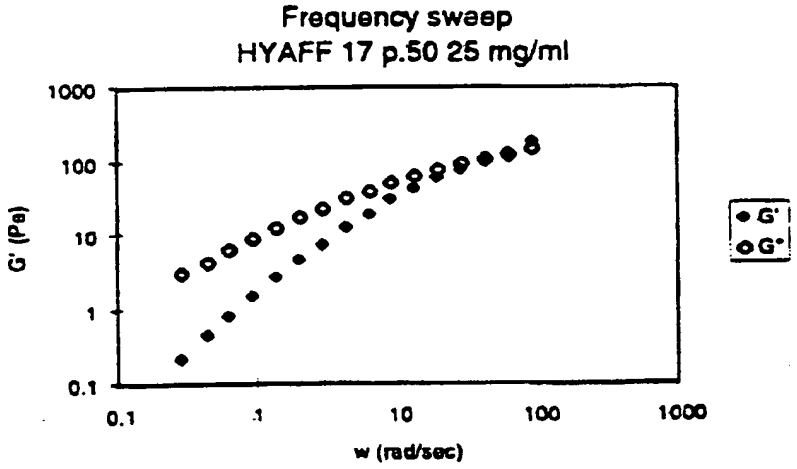


Figure 9

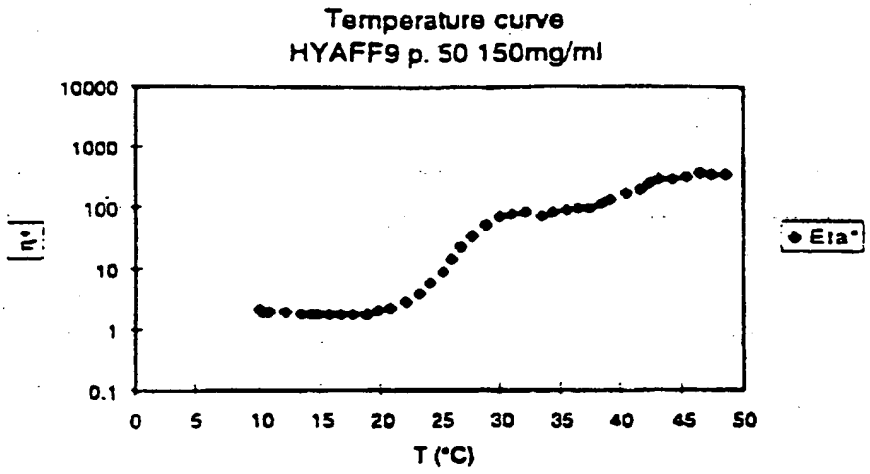


Figure 10

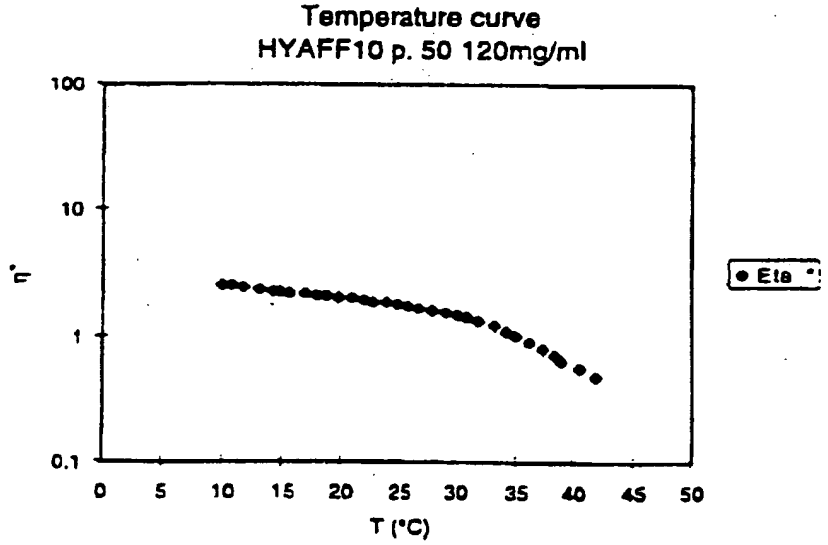


Figure 11

Temperature curve

HYAFF11 p. 50 50mg/ml

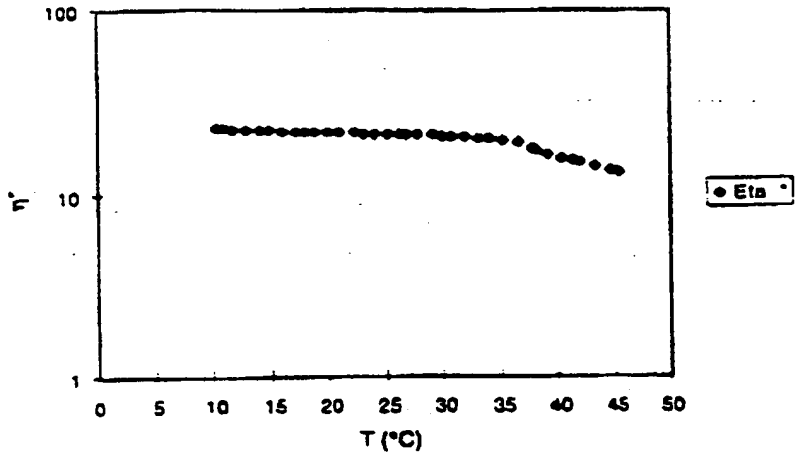


Figure 12

Temperature curve

HYAFF17 p. 50 20mg/ml

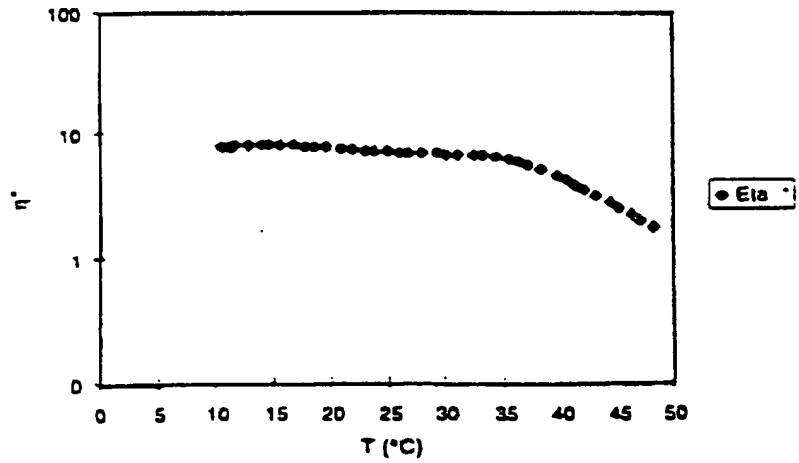


Figure 13

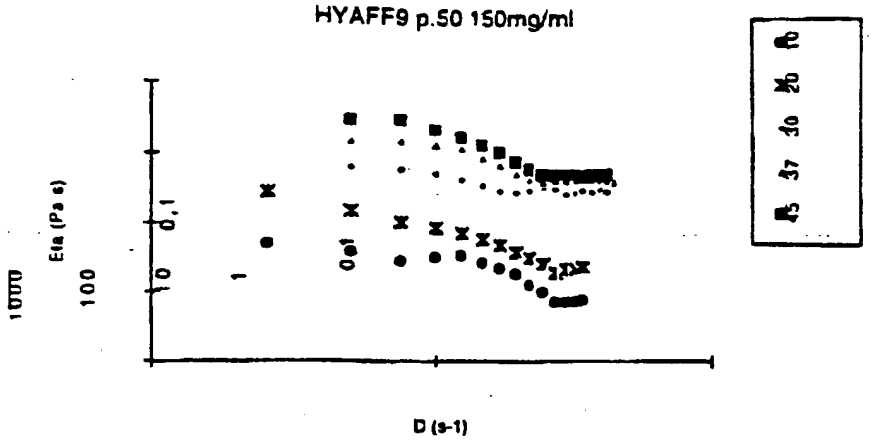
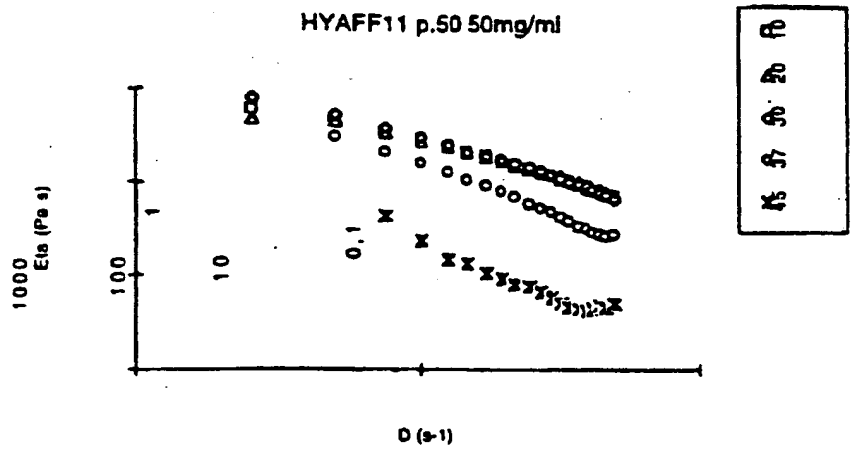


Figure 14



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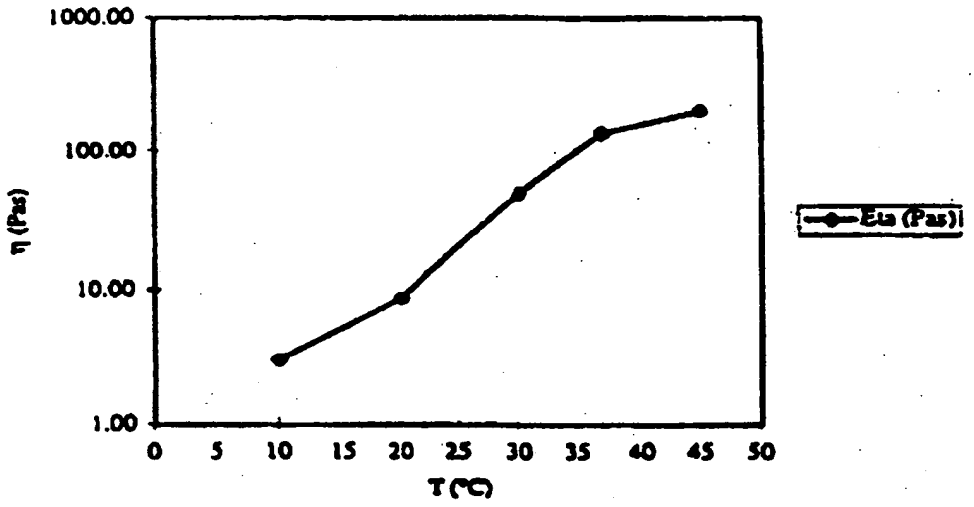

(57) Abstract

Viscoelastic compositions are described comprised of esters of hyaluronic acid which are uniquely advantageous because their viscoelastic characteristics may vary according to shearing stress and temperature.

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INTERNATIONAL SEARCH REPORT

Int. tional Application No
PCT/EP 98/07020

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/36 A61K9/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 216 453 A (FIDIA SPA) 1 April 1987 cited in the application see page 10 - page 19 see page 28, paragraph 4 - page 29, paragraph 1 see page 49; example 1 see page 54 - page 55; example 8 see page 69 - page 71; examples 24,26 see page 105, line 17 see claims 15-22,26,27 ---	1-4,6,7, 9-13
X,P	WO 97 49412 A (CALLEGARO LANFRANCO ;PAPARELLA ANNAMARIA (IT); BELINI DAVIDE (IT);) 31 December 1997 see page 1, line 8 see page 7, line 21 - page 8, line 9 see page 10, line 22 - page 12, line 27 see page 21 - page 22; example 8 --- -/--	1-4,10, 11,13

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
10 May 1999	25/05/1999

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Int .tional Application No
PCT/EP 98/07020

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indic :tion.where appropnate, of the relevant passages	Relevant to claim No.
X	WO 96 37519 A (FIDIA ADVANCED BIOPOLYMERS SRL ;BELLINI DAVIDE (IT); CALLEGARO LAN) 28 November 1996 see page 5, line 18 - page 6, line 27 see page 8, line 12 - line 19 see page 14 - page 15; examples 6-8	1,6,8-13
X	EP 0 341 745 A (FIDIA SPA) 15 November 1989 cited in the application see page 30 see page 16; example 9	1-13
A	DATABASE WPI Section Ch, Week 9739 Derwent Publications Ltd., London, GB; Class B04, AN 97-418785 XP002102269 & IT 1 268 702 B (FIDIA ADVANCED BIOPOLYMERS SRL), 6 March 1997 see abstract	1

3

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 98/07020

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 9-13 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [X] Claims Nos.: 1,9-13
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/EP 98/07020

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Claims Nos.: 1,9-13

The independent claims 1 and 9-13 do not meet the requirements of Article 6 PCT, in that the matter for which protection is sought is only defined in term of the result to be achieved. Since it is not clear from these independent claims which esters of hyaluronic acid with an alcohol presents the claimed properties, the search had to be restricted for economic reasons to the compounds claimed in the dependent claim7, to the compounds disclosed in the description on pages 2-4 and in the examples (PCT Search Guidelines PCT/GL2, Chapter III, 2.1., 3.6. and 3.7., Rule 33(3) PCT).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ational Application No
PCT/EP 98/07020

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